**A British Society for Haematology Good Practice Paper: Recommendations for Laboratory Testing of Patients with Acute Myeloid Leukaemia in the UK (Genetics Section).**

## Genetics

Comprehensive genetic profiling, comprising cytogenetic and molecular genetic testing, is integral to the diagnosis and classification of AML (refs). Detection of disease-specific abnormalities supports accurate diagnosis and yields prognostic information for risk stratification and is critical at diagnosis and at relapse. If a case is discovered to be high risk MDS (e.g. high blast count), then at the time of initial request or subsequently, testing should be undertaken as per the AML pathway.

Accurate and rapid genetic characterisation allows specific therapeutic interventions; either according to particular subtypes defined by their genomic architecture (e.g. CBF leukaemia, AML-MRC) or increasingly to specific genetic lesions (e.g. FLT3 and IDH inhibitors). Identification of characteristic genetic lesions also allows targeted molecular measurement of treatment response and residual disease. Furthermore, acquisition of the appropriate substrates for further studies is essential and cell suspension, DNA and RNA should be banked, with appropriate written informed consent in place, at diagnosis and relapse where possible.

Cytogenetic testing is mandatory at diagnosis and relapse for risk stratification and to support the rapid identification of those patients with AML-MRC, who may benefit from CPX-351. Conventional cytogenetic analysis (CCA) may be supplemented by rapid fluorescence *in situ* hybridisation (FISH) testing to promptly identify cardinal

lesions associated with various AML subtypes, however in the absence of a diagnostic finding FISH must always be supplemented by a rapid CCA. In the event of CCA failure, FISH for del(5q), del(7q) and monosomy 7 and 17p (TP53) will detect a significant proportion of abnormalities associated with AML-MRC, however, it should be noted that multiple rare abnormalities and complex karyotype would not be covered by this approach.

It is essential that RNA is stored in all cases at diagnosis as a baseline for later monitoring. This is particularly important to characterise the molecular breakpoints in PML-RARA, CBFB and KMT2A gene fusions for subsequent RQ-PCR. Monitoring by FISH or CCA is not routinely recommended, however may add value in clarifying atypical or discordant results of other testing modalities or where progression or relapse are suspected.

*PML-RARA* testing is not mandatory for all cases of AML, but must be rapidly initiated in selected cases where the results of other testing provide a high index of suspicion of APL. Where indicated PML-RARA testing must be available within 24 hours. It is important to note that a small proportion of rearrangements can occur which are both chromosomally cryptic and also undetected by FISH analysis. Molecular characterisation should be offered where clinical suspicion persists for the identification of cryptic abnormalities in difficult cases. The results of such supplementary studies must be available within 72 hours.

The characteristic lesions associated with CBF leukaemia should be identified within 72 hours of sample receipt. This can be undertaken by FISH, molecular

characterisation or a rapid karyotype. It is important to note that cytogenetically cryptic *CBFB* and *RUNX1-RUNX1T1* rearrangements must be excluded by an alternate method where a diagnostic suspicion of CBF leukaemia persists.

Testing for *KMT2A* rearrangement is essential, but less time critical than CBF, or FLT3, as it is unlikely to impact initial therapy decisions. If not undertaken as part of upfront screen, then *KMT2A* testing is mandatory for cases with a normal karyotype or where CCA fails to yield a result. Given the well documented heterogeneity of *KMT2A* partners then a gene partner agnostic approach such as 'breakapart' FISH testing, or an RNA-based next-generation sequencing (NGS) fusion approach is essential. Where a *KMT2A* rearrangement is identified by a partner agnostic approach it is essential for appropriate risk categorisation that further testing is undertaken to determine whether this represents a t(9;11); *MLLT3-KMT2A* fusion (Dohner 2017). This may be by CCA, metaphase FISH or molecular characterisation.

Internal tandem duplications (ITD) and tyrosine kinase domain (TKD) driver variants in *FLT3* have been shown to respond to FLT3 inhibitors and as such their rapid identification is critical. It is recommended that the *FLT3*-ITD allelic ratio (AR) is reported, although it is recognised that further efforts are ongoing to standardise measurement and reporting of AR. Whilst rapid identification of the recurrent driver variants in exon 11 of *NPM1* is not mandated, in practice they are often identified in tandem with *FLT3* analyses for prognostic purposes and can also aid interpretation of cases of suspected APL without *PML-RARA* rearrangement [Swerdlow 2017].

NGS panel testing for the identification of driver variants within *ASXL1*, *CEBPA*, *RUNX1*, *TP53*, *FLT3*, *IDH1* and *IDH2* is mandatory. The majority of NGS panels available for use in AML will also contain a range of genes associated with other myeloid diagnoses and the reporting of these is considered desirable, but not mandatory at this time. In the future, rapid diagnostics for these and other abnormalities may be required for selection of upfront therapy, however at present this is only relevant within specific clinical trials and should be provided by the respective trials laboratory where needed.

It is important to recognise that driver variants identified by somatic only testing may in fact be of germline origin, and protocols to robustly identify and confirm such variants in high-actionability germline cancer predisposition genes (i.e. *RUNX1, CEBPA, DDX41, ANKRD26, ETV6, GATA2*) are essential. The 2016 revision of the WHO classification of myeloid neoplasms (Arber et al 2016) now includes a section on myeloid neoplasms, including AML, with germ line predisposition. Laboratories must have processes in place to identify and report potential germline findings (Mandelekar 2019) in high-actionability genes from somatic only sequencing. Where a potential germline finding has been identified then a detailed clinical and family history are essential in determining whether to pursue further testing to confirm the aetiology of the variant. Should germline testing be indicated then a skin biopsy to obtain cultured skin fibroblasts will be required for further characterisation. Discussion and, depending upon local practice, referral of such cases to Clinical Genetics should be considered.

It is important to stress the importance of identifying a familial predisposition to haematological cancer, particularly to enable wider testing when considering transplantation from a related donor. A family history of MDS/acute leukaemia/aplastic anaemia, early onset of cancers of any type, or multiple close relatives with cancer should always be sought. In addition, a personal or family history of cytopaenias, abnormal bleeding, skin/nail abnormalities, idiopathic liver disease, immune defects, atypical infections, lymphoedema, limb abnormalities or pulmonary fibrosis should be elicited. Index patients reporting this history should be referred to Clinical Genetics for counselling and expert advice.

Conversely, in patients with a clinical or family history suggestive of an AML predisposition syndrome or inherited bone marrow failure disorder, up front germline testing may be warranted. Testing will typically be undertaken by large constitutional NGS panel analysis although, where there is a suspicion of Fanconi anaemia, then functional cytogenetic studies of mutagen (e.g. DEB, MMC) sensitivity may be appropriate, with further characterisation of any positive findings by molecular studies to inform wider family studies.

Whole genome sequencing (WGS) is an emerging technique in UK clinical practice which at present may be used to supplement standard of care studies. Over time however WGS offers the potential to replace and enhance standard of care diagnostics and as WGS pathways embed and expand and as the turnaround times for testing improve it is likely that this technique will supplant some of the current testing modalities.

## Table 1. Recommended turnaround times for genetic tests at diagnosis/relapse

| Test Name | Special Indication | TAT Days |
|---|---|---|
| *NPM1* exon 11 | | 3 |
| *FLT3* ITD § | | 3 |
| *FLT3* TKD hotspot § | | 3 |
| FISH/PCR *CBFB* [inv(16)] | | 3 |
| FISH/PCR *RUNX1-RUNX1T1* [t(8;21)] | | 3 |
| AML Karyotype §+ | | 3~7* |
| KMT2A FISH | | 14 |
| AML NGS Panel §* | | 14 |
| WGS Germline and Tumour | | 42 |
| FISH *PML-RARA* [t(15;17)] | Suspicion of APL on morphology or flow | 1 |
| RT-PCR *PML-RARA* [t(15;17)] | Suspicion of APL on morphology or flow. FISH inconclusive or negative | 3 |
| MyeChild01 FISH panel | Children and young adults, if no other primary genetic changes | 7 |
| MLDS panel (*GATA1*) | Children with known trisomy 21 | 21 |
| Fanconi breakage testing | Suspected Fanconi anaemia pre-transplant | 14 |
| Inherited bone marrow failure syndrome panel | Suspected familial predisposition syndrome | 84 |

§ It is essential that these tests are repeated at relapse. Other tests may need to be repeated at relapse, depending on the clinical situation.

+Cytogenetically cryptic CBF should be excluded where diagnostic suspicion persists

## Table 2. Recommended turnaround times for genetic tests post treatment / MRD

| NHSE Test Directory Code | Test Name | TAT (days) |
|---|---|---|
| M80.9/11-14 | AML Molecular MRD (*NPM1*mut and fusion genes) | 7-14* |
| M80.5 | AML FISH (if flow/molecular MRD marker absent) | 21§ |
| M80.3 | AML Karyotype (if flow/molecular MRD marker absent) | 21§ |

Urgent samples requiring a faster turnaround time (e.g. 7-10 days) include:

*NPM1* mutated patients at post course 2 time point (peripheral blood sample essential for risk stratification).

Repeat sample following a previous concerning result (e.g. suspicious for molecular relapse or molecular progression).

Clinical suspicion for relapse (e.g. falling counts).

**Genetics Recommendations in bold with bullet points**

- **Cytogenetic and molecular genetic analyses are critical at diagnosis and relapse**
- **Where indicated PML-RARA testing must be completed within 24hours of receipt**
- **Rapid identification of core binding factor leukaemia and FLT3 internal tandem duplication (ITD) and tyrosine kinase domain (TKD) is essential**
- **A complete cytogenetic workup is required within 7 days of sample receipt**
- **RNA must be stored at diagnosis, in all cases, to access baseline testing for future monitoring.**

## Table. Summary of new drug approvals in AML (2018-21)

| Drug | Drug Class | Approved indication* | NICE | FDA | EMEA |
|---|---|---|---|---|---|
| Midostaurin | Multi-targeted kinase inhibitor | Newly-diagnosed *FLT3*-mutated AML (in combination with intensive induction and consolidation chemotherapy, and alone after complete response as maintenance therapy) | Recommended (June 2018) | ✓ | ✓ |
| Arsenic trioxide | Inorganic compound | Acute promyelocytic leukaemia (APL).<br>1. Untreated, low-to-intermediate risk disease (WBC<10x$10^6$/ml) when given with all-trans-retinoic acid (ATRA) or<br>2.Relapsed or refractory disease, after a retinoid and chemotherapy. | Recommended (June 2018) | ✓ | ✓ |
| Gemtuzumab ozogamicin | Anti-CD33 antibody-drug conjugate | Untreated de novo CD33-positive AML in adults. Cytogenetics favourable, intermediate, failed or not yet available | Recommended (Oct 2018) | ✓ | ✓ |
| CPX-351 | Cytotoxic (liposomal daunorubin/cytarabine) | Untreated therapy-related AML or AML with myelodysplasia-related changes in adults. | Recommended (Nov 2018) | ✓ | ✓ |
| Gilteritinib | FLT3 inhibitor | Relapsed or refractory *FLT3*-mutation-positive AML in adults | Recommended (Aug 2020) | ✓ | ✓ |
| Enasidenib | IDH2 inhibitor | Relapsed/refractory IDH2-mutated AML | Not assessed | ✓ | × |
| Ivosidenib | IDH1 inhibitor | Relapsed/refractory IDH-1 mutated AML | Not assessed | ✓ | × |
| Venetoclax | BCL2 inhibitor | Newly-diagnosed AML (in combination with hypomethylating agent or low dose cytarabine) for patients unsuitable for intensive chemotherapy | Under assessment | ✓ | ✓ |
| Glasdegib | Smoothened inhibitor | Newly-diagnosed AML (in combination with low dose cytarabine) for patients unsuitable for intensive chemotherapy) | Not assessed | ✓ | ✓ |
| CC-486 | Oral hypomethylating agent | As maintenance treatment for adults who have achieved CR/CRi following intensive induction chemotherapy who are not proceeding to haematopoietic SCT | Under assessment | ✓ | ✓ |

## Table. Sample Requirements

| | | |
|---|---|---|
| **Genetics** | Cytogenetics and FISH | • BM recommended but PB may be sufficient if PB involved.<br>• 1-2 ml bone marrow in heparinised Transport Medium (supplied by Cytogenetics lab) or Lithium heparin tube. Should reach the laboratory within 24 hours. |
| | Molecular rapid single target testing | • 2-3 mL EDTA-anticoagulated bone marrow / or 4 mL EDTA-anticoagulated peripheral blood |
| | Myeloid gene panel analysis | • 2-3 mL EDTA-anticoagulated bone marrow / or 4 mL EDTA-anticoagulated peripheral blood |
| | Molecular fusion detection and MRD monitoring | • 2-3 mL EDTA-anticoagulated bone marrow or 15-20 mL EDTA-anticoagulated peripheral blood. Preferred substrate is bone marrow for RNA extraction which should reach the laboratory within 48 hours. |